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Effect of Liv.52 on Membrane Lipids in Carbon Tetrachloride-induced Hepatotoxicity in Rats

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Administration of CCl_4 to normal as well as Liv.52 fed rats increased *in vitro* formation of lipid peroxides; cholesterol and phospholipid composition of hepatic mitochondrial fraction was not altered, the recovery of cholesterol, phospholipid and protein in the microsomal fraction decreased and the microsomes became depleted in cholesterol, total and individual phospholipids. However, feeding of Liv.52 to rats gave marked protection against the increase in lipid peroxidation and decrease in the cholesterol and phospholipid contents of microsomal fraction.

Administration of CCl_4 is known to adversely affect the activity of some membrane-bound enzymes of hepatic mitochondrial[1,2] and microsomal fractions[3] and induce peroxidative degradation of mitochondrial and microsomal lipids[4]. It has also been shown that rats fed on vitamin E (antioxidant) rich diet showed resistance to liver injury by CCl4. Earlier work from our laboratory has shown that oral feeding of Liv.52 (Ayurvedic liver tonic, The Himalaya Drug Co., Bombay) to young weanling rats for 11 weeks gave protection against CCl_4-induced decrease in succinate dehydrogenase, cytochrome c oxidase and adenosine triphosphatase in the hepatic mitochondrial fraction and aniline hydroxylase and aminopyrine N-demethylase in post-mitochondrial supernatant fraction of rat liver. Feeding of Liv.52 per se decreased incorporation of ^{14}C-acetate *in vivo* in liver lipids, content of lysophosphatidyl choline and sphingomyelin decreased and that of phosphatidyl serine and phosphatidyl ethanolamine and tocopherols increased; *in vitro* lipid peroxidation by liver homogenate of Liv.52 fed rats was less than that in the control group[7].

In addition, there are numerous reports that Liv.52 gives protection against CCl_4-induced liver injury[8]. Therefore, it was considered of interest to examine if prolonged feeding of Liv.52 gave any protection against CCl_4-induced liver injury at the level of lipid composition of the mitochondrial and microsomal fraction as well as lipid peroxidation and antioxidant(s).

MATERIALS AND METHODS

Male weanling rats (Druckrey strain, CDRI animal house) were divided into four groups of 6 rats each. The rats of groups III and IV were orally given Liv.52 syrup (0.125 ml/kg body wt/day which is equivalent to human dose) for 11 weeks while corresponding amount of normal saline was given to the rats of groups I and II. Two rats were kept in one cage. All the rats received *ad lib* standard pellet diet (Hindustan Lever Ltd., Bombay). After 11 weeks of this regimen, sublethal injection of CCl_4 (0.7 ml/kg body wt. ip) was given for 2 successive days to rats of groups II and IV as described by Quazi-. After 48 hrs of the injection of CCl_4, the rats were killed by decapitation and livers were immediately excised out, washed with chilled saline. Homogenates of liver (10% w/v) were prepared in 1.15% KCl (w/v). Lipid peroxides (malonyl dialdehyde) formed in the homogenates after 3 hr incubation at 37°C were estimated according to Sharma and Krishna

Murti[10]. Total tocopherols in the homogenates were estimated according to Lehman. Mitochondrial and microsomal fractions were prepared according to Schneider and Hogeboom[12]. Lipids of mitochondrial and microsomal fractions were extracted according to Folch *et al*[13]. Total cholesterol in the lipids was estimated according to Zlatkis *et al*[14]. Phospholipids were resolved by TLC on silica gel G using chloroform-methanol-acetic acid-water (65:25:4:2 v/v) as solvent and identified by comparing their Rf values with those of simultaneously-run authentic standard phospholipids. Silica gel of the iodine-marked spots was scraped. Lipid samples or silica gel scrapings were digested with 70% PCA and phosphorus in the digests estimated according to Wagner *et al*[15].

Protein was estimated according to Lowry *et al.*[16] using bovine serum albumin as standard.

RESULTS AND DISCUSSION

Table 1 shows that the administration of CCl_4 to normal rats (group II) increased the formation of lipid peroxides *in vitro* by liver as compared to control (group I). However in Liv.52-fed rats which were not treated with CCl_4 (group III), lesser amounts of lipid peroxides were formed as compared to control (group I) and when CCl_4 was given to rats fed Liv.52 (group IV), the increase in lipid peroxidation was of a lesser magnitude than in normal rats treated with CCl_4 (group II). This shows that oral feeding of Liv.52 exerts protection against increased lipid peroxidation caused by administration of CCl_4. Level of tocopherols (anti-oxidant) in liver of rats given CCl_4 (group II) was lower than in the control rats (group I). Liv.52-fed rats (group III) showed an increase in the content of tocopherols in liver as compared to control (group I). Decrease in the tocopherol content of liver caused by the administration of CCl_4 was less pronounced in rats, which were fed Liv.52 (groups II and IV). Tocopherols are known to inhibit lipid peroxidation[17] and exert a protective effect against lethal dose of CCl_4[5]. Accordingly CCl_4-treated rats (group II) showed enhanced lipid peroxidation in liver along with decreased contents of tocopherols while Liv.52-fed rats which were given CCl_4 treatment (group IV) showed decreased level of lipid peroxidation along with an increase in tocopherol contents. This indicates that Liv.52 inhibits CCl_4-induced lipid peroxidation presumably by increasing tocopherol level.

Table 1: *In vitro* lipid peroxidation and tocopherols in liver (Values are mean ± SD from 6 rats in each group)

	Group I	Group II	Group III	Group IV
Lipid peroxidation*	2.78 ± 0.40	4.82 ± 0.40[a]	1.81 ± 0.50[a]	2.97 ± 0.80[b]
Tocopherols**	663 ± 50	543 ± 35[a]	846 ± 50[a]	725 ± 66[b]

*TBA value – OD at 535 nm/g wet tissue;
**µg tocopherol/g liver.
p values: [a]<0.01 as compared to group I;
[b]<0.01 as compared to group III.

The results of Table 2 show that percent recovery of membrane proteins, phospholipids and cholesterol in the mitochondrial fraction of liver of rats of all the 4 groups was more or less the same. It is clear from Table 3 that neither administration of CCl_4 nor feeding of Liv.52 had any effect on the levels of phospholipids, cholesterol and individual phospholipids of mitochondrial fraction.

Results of Table 2 also show that in CCl_4 treated rats per cent recovery of phospholipids, proteins and cholesterol in microsomal fraction of liver was considerably less than in control rats which indicates that microsomal membranes were degenerated during CCl_4-induced hepatotoxicity.

Table 2: Recovery of membrane-constituents in mitochondrial and microsomal fraction
(Values mean ± SD from 6 rats in each group, are actual recovery of the constituent from liver wet wt. Figures in parentheses are percent of constituent of total homogenate recovered in the subcellular fraction)

		Group I	Group II	Group III	Group IV
Protein (mg)	Mitochondria	36.53 ± 1.27 (23.02)	32.8 ± 3.64 (24.85)	39.57 ± 6.55 (24.83)	39.87 ± 5.29 (23.87)
	Microsome	21.00 ± 1.64 (13.82)	15.14 ± 1.73* (11.13)	20.11 ± 2.16 (12.91)	18.18 ± 0.60 (13.25)
Phospholipid (mg)	Mitochondria	5.39 ± 0.36 (18.11)	4.9 ± 0.47 (19.47)	5.59 ± 0.49 (19.15)	5.09 ± 0.26 (20.24)
	Microsome	2.63 ± 0.39 (9.18)	1.25 ± 0.23* (5.14)	2.57 ± 0.41 (8.84)	1.84 ± 0.37** (7.31)
Cholesterol (mg)	Mitochondria	1.28 ± 0.21 (17.18)	1.19 ± 0.18 (16.37)	1.35 ± 0.12 (19.94)	1.37 ± 0.26 (19.77)
	Microsome	0.95 ± 0.09 (11.55)	0.49 ± 0.03* (6.78)	0.91 ± 0.10 (13.31)	0.80 ±0.06** (11.64)

p values: *<0.01 compared to group I; **<0.01 compared to group III.

Table 3: Cholesterol and phospholipid composition of mitochondrial fraction
(Values are mean ± SD from 6 rats in each group and expressed for 1 mg mitochondrial protein. All changes are statistically not significant)

	Group I	Group II	Group III	Group IV
Cholesterol (µg)	35.00 ± 1.87	36.21 ± 2.75	34.48 ± 6.95	32.06 ± 1.72
Total phospholipids (µg)	144.42 ± 4.16	146.9 ± 5.03	141.6 ± 2.36	139.8 ± 4.25
Lysophosphatidyl choline (µg)	2.48 ± 0.32	2.76 ± 0.56	2.7 ± 0.53	2.74 ± 0.54
Sphingomyelin (µg)	5.94 ± 0.40	6.15 ± 1.57	5.73 ± 0.13	5.54 ± 0.85
Phosphatidyl choline (µg)	79.27 ± 4.35	79.89 ± 1.93	75.73 ± 2.48	77.97 ± 3.13
Phosphatidyl ethanolamine (µg)	29.17 ± 1.83	28.91 ± 0.81	28.08 ± 0.80	27.12 ± 0.49
Phosphatidyl serine (µg)	12.66 ±0.3	13.55 ± 1.0	12.88 ± 0.89	13.74 ± 0.76
Cardiolipin + Phosphatidic acid (µg)	8.01 ± 0.96	8.03 ± 1.37	8.25 ± 0.46	8.20 ± 0.41

Feeding of Liv.52 did not elicit any significant change in the recovery of these constituents in the hepatic microsomal fraction of control rats (groups I and III) but when CCl_4 was given to Liv.52-fed rats, the recovery of proteins and cholesterol increased to almost normal values while recovery to phospholipids was considerably improved (groups I and IV) as compared to groups I and II. This shows that feeding of Liv.52 gave protection against degeneration of endoplasmic reticulum in liver in CCl_4-induced hepatotoxicity.

Table 4 gives the cholesterol and phospholipid composition of hepatic microsomal fraction. It is evident from Table 4 that levels of phospholipids of cholesterol decreased in the hepatic microsomal fraction of rats treated with CCl_4 (groups I and II). Liv.52-fed rats (group III) did not exhibit any change in the phospholipids and cholesterol contents of the microsomal fraction (groups I and III) but when Liv.52 fed rats were given CCl_4 a marginal decrease in the phospholipid and cholesterol contents of microsomal fraction was observed (as compared to control rats). The levels of all the individual phospholipids except phosphatidyl ethanolamine registered a decrease in rats given CCl_4 (group II). Feeding of Liv.52 to control rats decreased the levels of lysophosphatidyl

choline and sphingomyelin and increased the levels of phosphatidyl serine. However when Liv.52-fed rats were given CCl_4 the decrease in phosphatidic acid was slightly protected.

Table 4: Cholesterol and phospholipid composition of microsomal fraction
(Values are mean ± SD from 6 rats in each group and expressed for 1 mg microsomal protein)

		Group I	Group II	Group III	Group IV
Cholesterol (µg)		45.48 ± 4.96	32.23 ± 2.18*	45.37 ± 5.26	42.52 ± 1.18
Total phospholipids (µg)		125.2 ± 1.38	82.78 ± 1.13*	128.3 ± 2.31	101.1 ± 3.6**
Individual phospholipids	Lysophosphatidyl choline (µg)	2.55 ± 0.1	1.53 ±0.04*	1.34 ± 0.38*	1.32 ± 0.13
	Sphingomyelin (µg)	5.86 ± 0.14	3.72 ± 0.13*	3.68 ± 0.32*	3.97 ± 0.34
	Phosphatidyl choline (µg)	67.61 ± 3.05	46.76 ± 2.40*	69.62 ± 6.88	61.44 ± 1.39
	Phosphatidyl serine (µg)	6.64 ± 0.34	4.98 ±0.28*	10.24 ± 0.71*	7.71 ± 0.70**
	Phosphatidyl ethanolamine (µg)	16.57 ± 1.19	16.16 ± 0.54	15.78 ± 1.40	15.75 ± 0.43
	Phosphatidic acid (µg)	6.10 ± 1.02	3.55 ± 0.05*	5.68 ± 0.43	5.05 ± 0.55

p values: *<0.01 compared to group I; **<0.01 compared to group III.

Recknagel and Ghoshal[18] demonstrated that during CCl_4 toxicity, free radicals are generated in or near the lipoidal centres of endoplasmic reticulum of hepatic parenchymal cells, this initiates autocatalytic peroxidative breakdown of microsomal lipids. The changes in the lipoidal elements of endoplasmic reticulum results in the morphological alterations of endoplasmic reticulum[19,20]; loss of drug metabolising activity[21] and loss of glucose-6-phosphatase activity[3]. In our previous communication[6] we have shown that administration of CCl_4 decreases the activities of drug metabolising enzymes and glucose-6-phosphatase which are associated with microsomal membranes and feeding of Liv.52 gave protection against the decrease in these enzymes. The present work demonstrates increase in the levels of lipid peroxides in liver of rats treated with CCl_4 followed by partial degeneration of hepatic microsomal fraction and alteration in its phospholipid and cholesterol composition. The enzyme changes observed in the microsomal fraction by giving Liv.52 or CCl_4 or both[6] appears to be due to alteration in the lipid composition and are at least partly protected by feeding of Liv.52 while changes in mitochondrial enzymes cannot be correlated with alteration in lipid composition or lipid peroxidation, but are nevertheless protected by Liv.52.

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